HALOGENATED COMPOSITION, METHOD FOR PREPARING SAME
AND USES THEREOF

Related Application

[0001]   This is a continuation of International Application No. PCT/FR02/00151, with an international filing date of January 16, 2002, which is based on French Patent Application No. 01/00862, filed January 23, 2001.

Field of the Invention

[0002]   This invention relates a new composition containing halogenated compounds for (1) the treatment of viral, bacterial, parasitical, fungal infections, or infections generated from non-conventional transmissible agents; (2) the treatment of chronic, progressive or acute inflammation; (3) immuno-modulator treatments, and/or tissue healing stimulator treatments; and (4) pre- and/or per- and/or post-surgical irrigations. The invention composition is particularly helpful as a local use antiseptic.

Background

1. The hypochlorite of alkaline metal.

[0003]   Hypochlorite of alkaline metal and, particularly, the sodium hypochlorite (NaOCl), has been used since the 19th century for its antiseptic properties. Alkaline metal hypochlorite is an alkaline metal salt of hypochlorous acid. The available chloride level of sodium hypochlorite solutions is equal to the addition of HOCl (hypochlorous acid) and OCl⁻ (hypochlorous anion) concentrations (Bloomfield & Miles, 1979). The hypochlorite active form, *i.e.*, the hypochlorous acid, is a highly strong oxidant that plays a role in the mammalian defense system. HOCl is synthesized in polymorphonuclear neutrophils and monocytes (Wright *et al.*,

1986) during the respiratory burst by the myeloperoxidase-H_2O_2-halide system. Hypochlorous acid is unstable and reacts readily with primary and secondary amines to generate various N-chloramines (Zgliczynski *et al.*, 1971).

[0004]   In polymorphonuclear cytosol and, especially in neutrophil cytosol, an amino acid (*i.e.* taurine) is particularly abundant and has a very high reactivity with hypochlorous acid to yield the taurine N-chloramine (TauCl). This chloramine is less toxic and reactive than hypochlorous acid. In addition, TauCl is the most stable of the chloramines (Zgliczynski *et al.*, 1971; Marquez & Dunford, 1994). Moreover, taurine seems to have a high protective role in both intra- and extra-cellular environments, *via* its high scavenger activity with hypochlorous acid (Cantin, 1994; J. Marcinkiewicz *et al.*, 1998). However, long-lived taurine N-chloramines can move and react (*i.e.*, oxidize and/or chlorinate) at distance from their formation to generate tissue damage (Zgliczynski *et al.*, 1971).

[0005]   At the physiological pH (7.4), taurine and hypochlorous acid react spontaneously and with a 1/1-molecule stoechimetry to yield a taurine N-monochloramine. At acidic pH, this reaction generates both taurine N-monochloramines and taurine (N,N)-dichloramines. Taurine and, particularly, nitrites (NO_2^-), compete with other antioxidants to scavenge hypochlorous acid in the extracellular medium. Their concentrations are roughly equal. Thus, the main hypo-chlorous acid scavengers are nitrites, which react together to yield a lesser toxic derivative than TauCl. In the polymorphonuclear neutrophil cytosol, due to its high concentration (≈ 20 mM), taurine is the main scavenger of hypochlorous acid (J. Marcinkiewicz, 2000).

2. Sodium hypochlorite, hypochlorous acid, N-chloramine properties.

    a. Dissolving tissue abilities.

[0006]   In aqueous solution, the sodium hypochlorite (NaOCl) is well known to be caustic. It is a non-specific agent able to hydrolyze necrotic tissues. This property is due to the

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presence of sodium hydroxide (NaOH). The tissue dissolving level (*e.g.* mainly necrotic tissues) is in accordance with NaOCl concentration, contact surface (Hand *et al.*, 1978), contact time and NaOCl solution amount used (The *et al.*, 1979).

[0007]   Thus, even if a NaOCl concentration lower than 0,5% is not good enough to totally dissolve necrotic tissues, the reduced toxicity of these low concentrations is interesting. However, this decreased ability to dissolve necrotic tissues may be made up for by a NaOCl temperature increased to 37°C, even if at this temperature, the NaOCl stability is below 24 hours.

b. HOCl and taurine N-monochloramine stability in aqueous solution.

· Sodium hypochlorite (NaOCl):

[0008]   Sodium hypochlorite is a highly unstable molecule. At levels below 5 g/l of available chlorine, its stability is under 2 weeks and depends on the following factors:

- Light: Sodium hypochlorite is highly sensitive to light and should be protected by suitable packaging.

- Temperature: NaOCl is highly sensitive to temperature greater than 30 °C.

- Presence of metal or organic matter: hypochlorite aqueous solution (containing HOCl molecules) (*i.e.*: $NaOCl + H_2O \Leftrightarrow HOCl + NaOH$) is neutralized by organic matter. Hypochlorite solution is efficient both when it can act readily and when it is in excess in comparison to an organic matter amount.

- pH value: EP 0471129 A1 has established that a pH value between 10 and 10.5 yields a high stability to NaOCl oxidative activity (greater than 24 months).

· Taurine N-chloramine:

[0009]   At a physiological pH (7.4) and at 37 °C, the taurine N-chloramine is the more stable of the chloramines (the oxidative activity decrease is below 5%/hour at 37°C) (Grisham MB, Jefferson MM, Melton DF, Thomas EL – J.Biol. Chem. 1984; 259: 10404-13). However, in

aqueous solution, the solubility of taurine N-chloramine sodium salt with a pH value between 7 and 8 is greater, but has a lower stability of its oxidative activities (DE 4041703 A1), and at a pH = 8.3, the stability decreases by around 30% in 15 days followed by a decrease of around 0.71% per day (*i.e.* this equals a decrease of around 61% in 65 days).

c. Cell toxicity and viability.

[0010] Cell toxicity results mainly from an intracellular protein loss, which generates both an adherence decrease to substrates and cell deformation.

[0011] Cell viability alteration results mainly from the irreversible decrease of mitochondrial activity and therefore, a reduction of energy generated by cell respiratory.

[0012] The vulnerability of different cell organisms to NaOCl and TauCl depends on many factors:

- The exposition level of the cell surface. Thus, cell systems with a high cell organization *e.g.* in epithelium and dental plaque are less sensitive (*i.e.* surface cells are sacrificed for profound cells) than one-cell systems (prokaryotes, mammalian mobile cells, or other one-cell systems).

- Membrane type that protects intracellular elements (*i.e.* membrane permeability level to oxidants). The most efficient are viral proteinic membranes.

- A membrane presence that protects key intracellular systems (*e.g.*, DNA (nucleus), energetic production (mitochondria), secretion process (Golgi's apparatus), *etc.*). Prokaryotes do not possess these protector systems and, consequently, are more vulnerable.

- The intracellular antioxidant amount (*i.e.*, gluthatione, acetyl N-cysteine, taurine, amino acids, thiol groups, etc.) that is specific for each cell type. Prokaryotes possess a down antioxidant level.

- The extracellular antioxidant amount (*i.e.* taurine, thiol groups, organic matter, metal, blood, extracellular matrix, *etc.*).

- The liquid flux level that irrigates cells and, consequently, dilutes oxidants.

- The exposition time to oxidants.

- The local physicochemical environment (*e.g.* surface-active, oxidants, olfactory or gustatory properties, pH, pKa, density, solubility, viscosity, coloration, water-ectanol sharing factor).

[0013]   In a therapeutic treatment *in vivo*, the factors described above should be integrated for the determination of active agent levels to adapt them to both clinic status and therapeutic aims.

i) Sodium hypochlorite (NaOCl) or hypochlorous acid (HOCl):

[0014]   On the rat macrophage like-cells RAW 264.7, with a (NaOCl) = 1 mM (NaOCl concentration), the cell viability is highly altered (irreversible)(Park E. *et al.*, 1997).

[0015]   On the mouse macrophages, with (HOCl) > 0.125 mM, cell death increases significantly. This toxicity is abolished by a nitrite (NO_2^-) excess (NO_2^- alone does not generate cytotoxic activity) (Marcinkiewicz J. *et al.*, 2000).

[0016]   On human macrophages, fibroblasts and keratinocytes, *in vitro*:

- With (NaOCl) = 13.433 mM, toxicity is so great that it cannot be neutralized by antioxidants (*i.e.* with physiological concentrations).

- With (NaOCl) > 6.7165 mM, NaOCl has a high toxicity.

- With (NaOCl) < 3.358 mM, toxicity can be neutralized by an antioxidant addition.

- With (NaOCl) < 1.679 mM, toxicity is very low with an antioxidant presence (Hidalgo E. & Dominguez C., 2000).

- The adherence loss of macrophages generated by HOCl: With (NaOCl) = 1.0075 mM, after two hours of contact *in vitro*, 95% of the cells are alive but only 40% keep their adherence to substrates.

[0017]   On human endothelial cells *in vitro* (Pullar JM *et al.*, 1999):

- With [HOCl] $\leq$25 µM, HOCl is not toxic.

- With [HOCl] > 25 µM, cell toxicity increases progressively (exposition time-dependent).

- With [HOCl] = 50 µM, some cell contractions were observed, the cells became rounded within the first 10 minutes and some lost their adherence after one hour and the majority after three hours.

[0018]   On human fibroblasts in vitro:

- With (NaOCl) $\geq$1,0075 mM (observed for 24 hours after a 15-minute exposition) cell viability is altered.

- With (NaOCl) = 16,791 mmol/l cell morbidity is complete.

- For 67,165 µmol/l < (NaOCl) < 671,655 µmol/l, 100% of cells are alive.

- With (NaOCl) < 671,655 µmol/l, and a FCS presence (2%), cell viability is not altered (24 hours of exposure) and both growth and cell proliferation are stimulated (the latter enhance with the (NaOCl) decrease and with a highest efficiency at 33,582 µmol/l) (Hidalgo E. & Dominguez C., Life Sci. 2000 Aug 4;67(11):1331-44).

- With (HOCl) < 50 µM, HOCl does not alter *in vitro* human fibroblast skin viability and does not induce cell apoptose (Vile G.F. *et al.*, 2000).

ii) The effects of taurine N-chloramine (TauCl) on cell viability:

[0019]   On rat C6 glioma cells, a (TauCl) = 0~2 mM does not alter cell viability *in vitro* (Liu Y. *et al.*, 1999).

[0020]   On human skin fibroblasts, a (TauCl) ≤ 100 µM does not induce cytotoxicity or cell apoptose *in vitro* (Vile G.F. *et al.*, 2000).

[0021]   On human synoviocytes-like fibroblastes, with (TauCl) = 400-500 µM, cell morphology changes (~30%–50% of cells took a rounded form and lost their adherence to the plastic surfaces) although viability has been preserved ($\geq 95\%$) (Kontny E. *et al.*, 1999).

[0022]   On mouse T cells:

- With (TauCl) = 30-300 µM, cell viability is not altered (*i.e.* mitochondrial activity).

- At 300 µM, TauCl is cytotoxic (Marcinkiewicz J. *et al.*, 1998).

[0023]   On mouse dendritic cells incubated 24 hours with TauCl:

- For 0.05 mM < (TauCl) < 0.5 mM, mitochondrial activity (cell viability) is not altered.

- With (TauCl) > 0,5 mM, cell viability decrease significantly (Marcinkiewicz J. *et al.*, 1999).

[0024]   On macrophages or macrophage-line cells, with a (TauCl) = 50~600µM, cell viability is not altered. (TauCl) > 1 mM alters it (Marcinkiewicz J. *et al.*, 1995).

     d. Cellular take-up of exogenous HOCl and taurine N-chloramine.

[0025]   HOCl is a lipophilic oxidant and, consequently, easily and readily cross cell membranes (*i.e.* ~80% of HOCl molecules are taken up by human fibroblasts within the first 10 minutes) (Vile G.F. *et al.*, 2000). *In vitro* with (HOCl) = 35 µM, endothelial cells take up 50% of HOCl molecules within ½ minute and 100% within 15 minutes, with a high majority within the first 10 minutes (Pullar J.M. *et al.*, Am J Physiol. 1999 Oct; 277(4 Pt 2): H1505-12).

[0026]   TauCl is taken up by specific transport systems. Therefore, *in vitro*, the K_m and the V_{max} values in relaxed rat RAW264.7 cells are 23.3 µM and 51.3 pmol/min/10^6 cells, respectively (K_m = 28,1 µM and V_{max} = 90,9 pmol/min/10^6 cells for taurine).

[0027]   In LPS-stimulated macrophages, K_m = 45,9 µM and V_{max} = 82,6 pmol/min/10^6 cells for TauCl, and K_m = 17,3 µM and V_{max} = 116,3 pmol/min/10^6 cells for taurine.

[0028]   Membrane transport systems are specific to each of these molecules and depend on Na^+ level, temperature, and energy.

[0029]   The blood biodistribution of TauCl and taurine induce a ready take up by cells of liver, lung, spleen, stomach, intestine and kidneys. In addition, cells present within an inflammatory site readily take up these two molecules (with a inflammation/blood ratio equal to 6.43 and 4.84 respectively) (Kim C. *et al.*, 1998). Others data show a ready take up by kidneys, liver, spleen, and marrow. The take up by heart and muscle is slow (Huxtable RJ, J. Nutr. 1981; 111:1275-86).

e. Antiseptic properties.

[0030]   Sodium hypochlorite is a very strong and efficient bactericidal, virucidal and fungicidal agent (Shih *et al.*, 1970; Bloomfield & Miles, 1979, Harrison & Hand, 1980). The bactericidal minimum concentration of NaOCl (*i.e.* for *Gram-* and *Gram+* bacteria) is 3.36 mM (0.025%) (Heggers J.P. *et al.*, 1991) and the minimum virucidal concentration for VIH is 19.062 mM (1%) of available chlorine.

[0031]   In contrast, TauCl has a very low bactericidal activity. Only dichloramines generate some bactericidal activity (*i.e.* with *E. Coli* in acidic conditions) (Marcinkiewicz J. *et al.*, 2000).

3. Inflammation.

[0032]   Inflammation is a defense mechanism toward all aggression types. Sentinel cells (*e.g.* macrophages and dendritic cells (DC)), that generate an immune system initialization *via* both a generation and a release of mediators detect an aggressor (Marcinkiewicz J. *et al.*, 1999). These mediators induce a reaction cascade and both activate and regulate the immune system in

an adaptive manner to the aggression type. After the aggressor agents are removed, a regulatory system generates an inflammation turnover followed by a healing/regeneration process.

[0033] Two immunity types are perceived: innate (natural) and acquired (adaptive).

[0034] The cell part of the innate (natural) immunity is made up of monocytes (mononuclear phagocytes), polymorphonuclear neutrophils (PMN), and natural killer cells (NK). These cells use the complement cascade, or some recognition protein, e.g., reactive protein C and amyloid protein. These proteins are able to attach themselves to carbohydrate molecules present on bacteria membranes. PMNs are included in the first mammalian defense line and cooperate closely with macrophages (one of the major effector cells of the immune system). PMNs are responsible for the non-specific defense in acute inflammation and macrophages take a similar role in both acute and chronic inflammations (Marcinkiewicz J. et al., 1994).

[0035] The acquired (adaptive) immunity involves several T cell types and uses antibodies as effector proteins. T cell receptors and antibodies are recognition molecules. B cells recognize carbohydrates, proteins, and some simple chemical structures while T cells recognize only peptides.

[0036] Dendritic cells (DC) play an important role. Under inflammatory mediator action, DCs migrate from non-lymphoid tissues to lymphoid organs where they lose their ability to scavenge antigens and acquire an increasing ability to stimulate T cells (Marcinkiewicz J. et al., 1994).

4. Inflammatory mediators.

[0037] Cytokines are the most important intercellular messenger molecules of the immune system (Megarbane B. et al., 1998). Cytokines are generated and released from activated immune cells and they induce some particular biological activities after binding to a specific target cell receptor, in an autocrine or a paracrine manner. Macrophages and T cells are

main productive cells of cytokines, although many other cells also can produce them. Cytokines are main and real regulators of both humoral and cellular immune response. Cytokines travel together and the balance of their activities is crucial for immune system regulation, *e.g.*, *via* a competition between TH1 (IL-2, INF-γ, TNF-β and IL-12) and TH2 (IL-4, IL-5, IL-10 and IL-13) T cells.

[0038]   TH1 cells are involved in cell immunity and are responsible for cytotoxic activities of macrophage, T cells and natural killer cells.

[0039]   TH2 cells are associated with humoral response, and, for example, IL-10 (*i.e.* a TH2 type cytokine) strongly inhibits effective functions of macrophages and TH1 cells (Marcinkiewicz J., 1997).

[0040]   Cytokine regulatory functions can be extended to a selection of immunoglobulin isotypes during humoral response. Thus, selective inhibitions of cytokines generate an immune response modulation.

[0041]   Eicosanoids (prostaglandins and leukotrienes) and nitric oxide (NO), produced by activated macrophages, have an important role in the regulation of cytokine production. Eicosanoids are generated from arachidonic acid, which is derived from cell membrane phospholipides.

[0042]   Prostaglandines (PG) are generated under the cyclooxygenase (COX) catalyzing action. Two cyclooxygenase types are distinguished: the constitutive form (COX1) and the induced form (COX2). COX2 production is activated within inflammatory cells by pro-inflammatory mediators. Thus, COX2 catalyzes the synthesis of prostaglandins E_2 (PGE$_2$) and prostacyclins I_2 (PGI$_2$) in macrophages, and prostaglandines D_2 in mast cells.

[0043]   Prostaglandins (particularly PGE_2) and leukotrienes (particularly LTB_4) change immune responses. Therefore, equilibrium in both production and effects of these eicosanoids is needed to induce a harmonious functioning of the immune system.

[0044]   Nitric oxide (NO) is synthesized from L-arginine under the catalyzing action of the constitutive nitric oxide synthetase ((cNOS) that is calcium dependent) or the induced nitric oxide synthetase ((iNOS) that is calcium independent).

[0045]   cNOS permits the synthesis of the basic form of nitric oxide (NO) in cells of both endothelium and nervous system.

[0046]   iNOS is found in a variety of cells including macrophages, neutrophils and hepatocytes. NO generation plays an important role in macrophage cytotoxicity and their ability to kill pathogen microorganisms and, consequently, in mammalian non-specific defense against many pathogens and tumor cells.

[0047]   More characteristics of these inflammatory mediators are described in Knight JA *et al.*, 2000; Marcinkiewicz J. *et al.*, 1997; and Megarbane B *et al.*, 1998.

5. The influence of hypochlorous acid and taurine N-chloramine on an inflammatory site.

· On bacteria.

[0010]   Rat peritoneal macrophages stimulated by non-chlorinated *Gram+* bacteria (*Staphylococcus aureus, S. epidermidis, and Escherichia coli*) release high concentrations of nitric oxide, TNF-α, and IL-6. The same bacteria chlorinated by HOCl lose their abilities to induce a nitric oxide and TNF-α release while IL-6 production and phagocytosis are not altered (Marcinkiewicz J. *et al.*, 1994).

· On endothelium.

[0049]   HOCl increases the endothelium permeability and promote leukocyte adherence to microcirculation endothelium.   Taurine N-chloramine reduces an endothelium permeability increase generated by PMN activities. Taurine alone is without effect (Tatsumi & Flies, 1994).

· On cellular growth.

[0050]   *In vitro*, on endothelial cells of the human umbilical vein, a HOCl down level (5 nM/1.2×10^5 cells) does not induce a cell death but a temporary stop of cell growth (Vissers MC *et al.*, 1999). In addition, low concentrations of both HOCl and physiological chloramines lead *in vitro* to an inhibition of DNA synthesis and cell division on skin fibroblasts (Vile GF *et al.*, 2000).

· On non-free proteins (*e.g.* collagen, *etc.*).

[0051]   HOCl is a very strong oxidant. In addition, HOCl chlorinates proteins and makes them more vulnerable to an endopeptidase-degradation. · Thus, HOCl contributes to a destruction of the tissue surrounding the inflammatory site. TauCl is an oxidant with lower strength and seems to have a lesser responsibility for damage to these tissues.

· On collagenases.

[0052]   TauCl induces a direct inhibition/inactivation of collagenases while it has no effect on the collagen proteolytic susceptibility.   In comparison, leucine and alanine N-monochloramines have no inhibitory effect on collagenases and increase the proteolytic susceptibility of collagen (Davies JMS *et al.*, 1994).

· On free proteins (ovalbumin, bacterial enzymes, etc.). ·

[0053]   Free protein chlorination enhances their immune sensitivity, likely *via* an improvement of both their treatment and presentation by antigen-presenting cells (*i.e.* macrophages and dendritic cells). This chlorination is ten times more important for HOCl than

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taurine-N-monochloramines (TauCl) but, *in vivo*, TauCl is more stable and, consequently, TauCl can be regarded as the main physiological chlorinating agent (Marcinkiewicz J. *et al.*, 1999).

· On dendritic cells (DC) (Marcinkiewicz J. *et al.*, 1999).

[0010]   Two hours pre-incubated rat DCs with TauCl underwent a concentration-dependent inhibitory activity. Thus, a TauCl concentration equal to 500 µM ((TauCl) = 500 µM) almost completely inhibits the DC release of reactive oxygen agents (ROS) generated *via* a respiratory burst, nitric oxide, PGE_2, TNF-α, IL-6, IL-10, and IL-12. In addition, the lipopolyssacharide-induced expression of MHC type II and molecule B7-2 is also inhibited. At this concentration, TauCl may be toxic to DC when they are exposed for a long time. With (TauCl) = 250 µM, TauCl has a more selective action. Therefore, it inhibits the production of IL-10, IL-12, PGE_2, and nitric oxide. TNF-α and ROS generation is not inhibited. In addition, a DC exposition to TauCl seems to promote a TH1 response and decreases the TH2 activity.

· On T cells.

[0055]   TauCl inhibits the release of IL-2 and IL-6 by T cells pre-incubated with a (TauCl) = 100-300 µM and stimulated with either a mitogen, an antigen or an ovalbumin-APC complex (Marcinkiewicz J. *et al.*, 1998).

· On phagocytes.

[0056]   Antigens chlorinated by HOCl or TauCl do not induce an production of inflammatory mediators by the phagocytes that phagocytosed these antigens (Marcinkiewicz J. *et al.*, 1994 & 1997).

· On macrophages.

[0010]   Chloramines such as taurine N-mono and (N,N)-dichloramine, N-monochloro-ethanolamine and N-dichlorophosphoethanolamine as well as NaOCl (sodium hypochlorite), all inhibited the release of nitric oxide in a dose-dependent manner. Serine N-chloramine (SerCl)

had a lesser half-life than TauCl (immediately after its preparation, (SerCl) = 300 μM inhibited the nitric oxide generation for 85%; after 24 hours, this inhibition was reduced to 22%). TauCl inhibited the oxide nitric generation for 98% with (TauCl) = 600 μM and 8-22% with (TauCl) = 100 μM (*i.e.*, this value changes with cell type). This inhibitory effect was executed within the iNOS gene transcription. Taurine alone was without effect (Marcinkiewicz J. *et al.*, 1995). HOCl (likely *via* TauCl activity) and TauCl inhibited COX2 post-transcriptional expression *i.e.* four-hours delay on the kinetic expression of mRNA (and consequently the PGE$_2$ production) and TNF-α transcriptional velocity (*i.e.*, in a dose-dependent manner with an IC$_{50}$ = 400μM)(Quinn MR *et al.*, 1996). TauCl inhibits COX2 expression either in non-stimulated and INF-γ-stimulated macrophages. In contrast, in INF-γ-stimulated macrophages TauCl inhibits both the iNOS expression and the production of TNF-α and IL-6. TauCl had no effect on IL-1α production for all stimulation levels. The native taurine alone had no effect on cytokine production. In addition, HOCl-oxidized plasma lipoproteins had an ability to reduce iNOS mRNA synthesis and, thus, to inhibit the nitric oxide production and contribute to atherosclerotic lesion development (Moeslinger T *et al.*, 2000).

· On polymorphonuclear neutrophils.

[0010]   TauCl inhibits production of nitric oxide, PGE$_2$, IL-6 and TNF-α in a dose-dependent manner. Native taurine has no effect. Some experiments (Marcinkiewicz J *et al.*, 1998 & 2000) with luminol chemiluminescence-dependent (LCL) measures have shown the following:

- Both taurine and TauCl reduced ROS production. However, only high taurine concentrations altered LCL and taurine activity is lower than TauCl.

- HOCl reduces myeloperoxidase activity in a retroactive dose-dependent manner. *In vitro*, TauCl and HOCl inhibit myeloperoxidase extracted from neutrophils.

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- HOCl (250 μM) inhibits hydrogen peroxide production in a dose-dependent manner. Taurine (500 μM) or nitrite (250 μM) neutralizes this inhibition. TauCl has no effect on this production.

- HOCl and TauCl induce a chemiluminescence dose-dependent decrease, TauCl (IC_{50} = 550 μM) is less efficient than HOCl (IC_{50} = 100 μM).

[0059]   TauCl and taurine inhibit superoxide anion (O_2^-) production by stimulated neutrophils. This inhibition involves a different mechanism than those implicated in TauCl formation (i.e., association of the taurine (or TauCl) with a myeloperoxydase specific inhibitor generates a synergic effect).

[0060]   However, high concentrations of taurine alter LCL. This activity is less important than TauCl (Marcinkiewicz J et al., 1998).

· On polymorphonuclear eosinophils.

[0061]   HOCl inactivates sulfidopeptide LTC4 sulfoxides and 6-trans-LTB4 leukotrienes only in an extracellular environment (Owen WF et al., 1987).

· On rat glioma cells C6.

[0062]   In the central nervous system of activated glioma cells, TauCl inhibits production of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-2 (MIP-2) both in dose-dependent and post-transcriptional manners (Liu Y et al., 1999). In addition, TauCl inhibits both the iNOS gene transcriptional expression (i.e., nitric oxide production) and the COX2 expression (i.e., PGE_2 production) via a post-transcriptional mechanism (Liu Y et al., 1998).

· On fibroblasts.

[0063]   In rheumatoid arthritis patients, TauCl inhibits fibroblast-like synoviocyte proliferation and decreases the activity of major transcriptional factors of both IL-6 (IC_{50} ~ 225

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μM) and IL-8 (IC_{50} ~ 450 μM) in a dose-dependent manner. Thus, TauCl reduces both IL-6

proinflammatory action and immune cell ability to migrate within an inflammatory site (*via* an

IL-8 inhibition). Whereas IL-6 inhibition is independent of the fibroblast stimulating agent used

(*e.g.* TNF-α, IL-1β or IL-17), IL-8 inhibition is dependent on the stimulation *via* TNF-α or IL-1β,

but not *via* IL-17. This shows different signaling pathways from TNF-α/IL-1β and IL-17

triggered-transduction (Kontny E *et al.*, 1999). These signaling pathways are dependent on two

transcription factors: NF-κB and AP-1. In addition, TauCl inhibits both spontaneous and bFGF-

stimulated syno-viocyte proliferation (Kontny E *et al.*, 2000).

[0064]   Low levels of both HOCl and physiological chloramines (NH_2Cl, TauCl and N-

chlorinated α-amino acid) inhibit both DNA synthesis and cell division of cultured human skin

fibroblasts (Vile GL *et al.*, 2000).

· On transcription factors NF-κB and AP-1.

[0065]   NF-κB-dependent gene expression may be altered by TauCl activity. In IL-1β-

stimulated human synoviocytes, transduction TauCl-inhibition of IL-6 and IL-8 is executed *via* a

DNA-bonding ability reduction of NF-κB and AP-1. IL-6 transcription is under a NF-κB

control, while both NF-κB and AP-1 control IL-8 transcription. Thus, a (TauCl) = 250 μM

selectively reduces the DNA-bonding of NF-κB (*i.e.*, the IL-6 transcription) without altering AP-

1 DNA-bonding (*i.e.*, the IL-8 transcription). TauCl acts on both NF-κB and AP-1 transcription

factors to inhibit the IL-6 and IL-8 transduction. At 500 μM, TauCl decreases the DNA-bonding

activity of both NF-κB and AP-1 (*i.e.*, the transcription of IL-6 and IL-8 is reduced)(Kontny E *et

al.*, 2000). These two transcription factors are regulated *via* a redox mechanism ((Sen C.K.,

Packer L., Fased J. 1996; 10:709-20), (Li N. & Karin M., Fased J. 1999; 13:1137-43), (Kunsch

C. & Medford R.M., Circ Res. 1999 Oct 15; 85(8):753-66.)). It seems that TauCl may interfere the intracellular redox status of these transcription factors and, therefore, some anti-inflammatory properties may be suggested from TauCl (Kontny E *et al.*, 2000).

· On complement.

[0066]   The C_5 component of the human complement may be activated by oxidants, *e.g.*, hydroxyl radicals, hypochlorite or chloramines (*i.e.*, TauCl and mainly NH_2Cl). This activation is due to a C_5 structural change induced by a Met. residue oxidation within the C_5 protein without peptide cleavage. These changes lead to a C_6 bonding site expression, which normally is formed after a C_5 specific cleavage in C_{5a} and C_{5b}, *via* one of two C_3/C_5 convertases. The C_5-oxidation product is similar to C_{5B}. Thus, it is able to initiate the combination of the C_{5-9} membrano-lytic complex.

[0067]   Chemotactic fragments are not directly generated, but activated C_5 components (like C_{5b}) are readily attacked by enzymes such as kallikrein, which produce C_{5a}-like fragments that have a chemotactic activity. It is likely that the C_{567} complex generated with C_5 also have a chemotactic activity (*i.e.*, similarly to C_{5b67} complex). In addition, the C_{5b-9} complex is known to stimulate PMNs at non-toxic concentrations. Thus, the same property may be suggested for the corresponding C_{5-9} complex and, consequently, this may lead to a vicious circle that increases tissue lesions (Vogt W, 1996).

Summary of the Invention

[0068]   This invention relates to a pharmaceutical composition including at least one halogenated compound, and at least one N-halogenated derivative of at least one compound selected from zwitterionic and/or amino acid compounds, where the composition does not generate substantial stimulation of myeloperoxidase activity in a mammal.

[0069]   This invention also relates to a method of preparing a pharmaceutical composition including mixing at least one halogenated compound and at least one zwitterionic compound and/or at least one amino acid or their derivatives, and optionally at least one excipient to obtain at least one N-halogenated derivative, and at least one halogenated compound in a sufficient therapeutic amount to not substantially stimulate myeloperoxidase activity in a mammal.

[0070]   This invention further relates to a method for treatment and/or preventing viral infections, and/or bacterial infections, and/or parasitical infections and/or fungal infections and/or diseases generated from non-conventional transmissible agents, in humans or animals including administering to a human or animal a pharmaceutically effective amount of a pharmaceutical composition including at least one halogenated compound, and at least one N-halogenated derivative of at least one compound selected from zwitterionic compounds and/or the amino acids or their derivatives without substantial stimulation or myeloperoxidase activity in the human or animal.

Detailed Description

[0071]   I have discovered that in inflammatory sites, beyond any bactericidal activity, NaOCl contributes to (1) an increase in the transition to the cleansing of necrotic and suppurating mass, (2) stimulates local immunity and (3) activates the tissue regeneration process. These abilities are induced from sodium hypochlorite (*i.e.*, hypochlorous acid (HOCl) properties and the hydrolysis generated from sodium hydroxide (NaOH)) and its N-chlorinated derivatives.

[0072]   Consequently, this invention provides a pharmaceutical composition comprising (i) at least one halogenated compound and (ii) at least one N-halogenated derivative of at least one compound selected from zwitterionic and/or amino acid compounds.

[0073]   Within compositions according to the invention, the halogenated compound (i) is an antiseptic.

[0074]   Amino acids included in the constitution of compositions according to the invention can be natural amino acids, derivatives or analogous of the latter.

[0075]   More particularly, the halogen of the (i) halogenated compounds and the (ii) N-halogenated derivatives of the invention composition, similar or different, may be fluorine, iodine, bromine, and mainly chlorine.

[0076]   Favorably, the halogenated compound (i) is an alkaline metal hypochlorite, and preferably the sodium hypochlorite, and the N-halogenated derivative (ii) is an N-halogen derivative of taurine and preferably a taurine N-halo-amine and even more preferably taurine N-chloramine.

[0077]   The invention composition is remarkable from its robust properties such as large spectrum of application such as anti-inflammatory, immunity modulation, and tissue healing stimulation as well as those without stimulation of myeloperoxidase activity.

[0078]   The hypochlorite titer of the invention composition is preferably below or equal to about 1 mole/liter of available chlorine, and can be adapted to clinical use. Usefully, the invention composition contains a hypochlorite of alkaline metal. Preferably, the invention composition contains a sodium hypochlorite *q.s.* with a minimum titer of available chlorine that is greater than or equal to about 1 picomole/liter.

[0079]   The N-chloramine titer of the invention composition is preferably less than or equal to abut 5 moles/liter, and may be adapted to clinical use. Usefully, the invention composition contains an N-halogenated derivative, such as the taurine N-chloramine, with a concentration between about 5 moles/liter and about 0.01 femtomoles/liter. Preferably, the

invention composition contains a N-halogenated derivative such as the taurine N-chloramine, *q.s.* with a minimum titer greater than or equal to about 0.01 femtomoles/liter.

[0080]   The (i) halogenated compound and the (ii) N-halogenated derivative are associated in the composition according to the invention with an excipient, such as purified water, in accordance with therapeutic use. Preferably, it concerns an osmotic (isotonic) purified water. This excipient may contain diverse agents, pharmaceutically compatible with both (i) the halogenated compound and (ii) the N-halogenated derivative, and which can allow for modification of some physicochemical properties such as stability, pH, pKa, density, solubility, viscosity, coloring, water/ectanol sharing factor, and surface-active, oxidative, olfactory, or gustatory properties of the invention composition *via* a suitable agent addition. The invention composition may also contain some anti-oxidants and/or amino acids that have a dilution effect *via* neutralization of some alkaline metal hypochlorite molecules. These anti-oxidants, amino acids and their N-halogenated derivatives should have a neutral pharmacological activity or its activity should be pointed to therapeutic aims and should not exercise a direct stimulation of myeloperoxidase activity in the presence of invention composition active agents.

[0081]   The invention also concerns the preparation of the composition described above. Thus, this composition can be sold in a form to prepare before use, *i.e.*, (i) the halogenated compound(s) can be mixed with (ii) the N-halogenated derivative(s) and one or several excipients. This presentation form can be considered if it is required to guarantee the best time stability of the composition and, in particular, the active agents that constitute the latter. However, even in a presentation where the constituting products would be associated, the invention composition can be sold with an excipient, such as purified water according to the therapeutic use. Preferably, this should be an osmotic (isotonic) purified water. In addition, this excipient may contain diverse agents pharmaceutically compatible with the totality of final

composition molecules, which allow for the modification of some physicochemical properties of the invention composition *via* an addition of suitable agent(s) such as stability, pH, pKa, density, solubility, viscosity, coloring, water/ectanol sharing factor, and surface-active, oxidative, olfactory, or gustatory properties.

[0082]   The invention composition can also be prepared before its administering to the patient *via* a mixture comprising:

(i)   at least one halogenated compound, and

(ii)   at least one N-halogenated derivative of at least one compound selected from zwitterionic and/or amino acid compounds, and their derivatives.

[0083]   More particularly, the halogen(s) of the halogenated compound (i) and the N-halogenated derivative (ii) may be selected from fluorine, iodine, bromine, and/or chlorine, most preferably chlorine.

[0084]   Favorably, the halogenated compound (i) is a halide such as an alkaline metal hypochlorite, and preferably the sodium hypochlorite, and the N-halogenated derivative (ii) is a taurine N-halogenated derivative and preferably a taurine N-haloamine and even more preferably the taurine N-chloramine.

[0085]   The aforementioned halogenated compound(s) (i) are usefully displayed in a liquid or semi-liquid (such as a gel) solution form, favorably within an excipient as described below. These solutions, advantageously hypochlorite solutions, may be stabilized in accordance with the patent EP 0 471 129 A1 *via* a pH regulatory agent to generate a pH between 10 and 10.5 with respect to cell viability.

[0086]   The aforementioned N-halogenated derivative(s) (ii) are usefully displayed in a liquid or a semi-liquid (such as a gel) solution form, favorably within an excipient as described below.

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[0087]   Favorably, the invention composition may be prepared *via* a mixture of the two solutions described above with at least one excipient according to therapeutic use such as purified water. It preferably contains the osmotic (isotonic) purified water. In addition, this excipient can contain diverse agents, pharmaceutically compatible with all molecules of the final mixing to modify some physicochemical properties of the invention composition such as stability, pH, pKa, density, solubility, viscosity, coloring, water/ectanol sharing factor, and surface-active, oxidative, olfactory, or gustatory properties *via* an addition of suitable agent(s).

[0088]   In addition to the process described above, the invention composition may be prepared *via* a mixture of the two following solutions:

(i)   at least one halogenated compound as defined above, which is usefully displayed in a liquid or a semi-liquid (such as a gel) solution form, preferably within an excipient as described above,

(iii)   at least one zwitterionic compound and/or at least one amino acid and/or at least one primary or secondary amine, (the zwitterionic compound and/or amino acid and/or primary or secondary amino amine are later referred to as "Zw/Aam"), which is usefully displayed in a liquid or a semi-liquid (such as a gel) solution form, favorably within an excipient as described above,

to obtain an association of both (i) at least one halogenated compound and (ii) at least one N-halogenated derivative, and this with a sufficient therapeutic amount of molecules to inhibit myeloperoxidase activity.

[0089]   This mixture is preferably realized with an excipient as defined above.

[0090]   In case Zw/Aam is an amino acid, it preferably concerns taurine or a taurine pharmaceutical analog.

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[0091]   In this realization method, when the antiseptic halogenated compound (i) is a halide such as alkaline metal hypochlorite (which is an alkaline metal salt of hypochlorous acid), derivatives generated will be N-chlorinated, and these will more particularly be N-chloramines.

[0092]   The hypochlorite titer of the first active solution (i) should take into consideration the stoichimetry and reactivity level of the reaction between hypochlorous acid and Zw/Aam molecules. In case this reaction is not complete, remaining Zw/Aam molecules should not stimulate myeloperoxidase activity in the presence of invention composition active agents.

[0093]   In case the stoichimetry is 1/1 and with a complete reaction (e.g., between hypochlorous acid and taurine), the hypochlorite titer of the first active solution is preferably lower than or equal to about 6 moles/liter of available chlorine, and must be adapted both to the Zw/Aam molecule amount of the second solution and to clinical status. In this preparation method, the halide solution (i) favorably contains an alkaline metal hypochlorite. Even more preferably, the haloid solution (i) contains sodium hypochlorite q.s. with an available chlorine titer between abut 6 moles/liter and about1,000.01 femtomoles/liter. The taurine titer of the second solution (iii) of this invention preparation method is preferably lower than or equal to about 1 moles/liter and may be adapted to clinical use. It is useful for the second solution (iii) of this invention preparation method to have a taurine concentration between about 5 moles/liter and about 0.01 femtomole/liter. Even more preferably, the second solution (iii) of this preparation method has a taurine titer greater than or equal to about 0.01 femtomole/liter.

[0094]   The excipient(s) preferably added in methods described above may be used as a secondary diluting solution with the aim to adapt the treatment to the clinical status. It usefully concerns osmotic (isotonic) purified water. This excipient will favorably be similar to the excipient used for the compounds and derivatives that have been mixed, and if they are not identical, the excipient should be pharmaceutically compatible to be mixed with the other

excipient(s), before all clinical uses. In addition, this excipient can contain diverse agents, pharmaceutically compatible with all molecules of the final therapeutic mixture with the object of modifying some physicochemical properties of the invention composition such as stability, pH, pKa, density, solubility, viscosity, coloring, water/ectanol sharing factor, and surface-active, oxidative, olfactory, or gustatory properties *via* an addition of a suitable agent(s).

[0095]   This excipient may contain anti-oxidants and/or amino acids that will have both a dilution effect and an oxidant neutralization of the active solution (i) (*e.g.*, the alkaline metal hypochlorite). These anti-oxidants, amino acids and their halogenated derivatives should have a neutral pharmaceutical activity or a pharmaceutical activity inducing the desired therapeutic effect. In all cases they should be both less toxic than the oxidants of (i) the main active solution and pharmaceutically compatible with all molecules of the final therapeutic solution.

[0096]   The composition according to the invention can also be sold in a form adapted to local use, *e.g.*, a gel or an aerosol.

[0097]   The above-mentioned invention composition is particularly useful in humans or animals for treatments of viral infections and/or bacterial infections and/or parasitical infections and/or fungal infections and/or diseases generated from non-conventional transmissible agents; and/or for treatments of chronic, progressive or acute inflammation; and/or for immunity modulator treatments; and/or for tissue regeneration stimulator treatments. In addition, the therapeutic composition may be used in *pre*-surgical irrigations and/or *per*-surgical irrigations and/or *post*-surgical irrigations.

[0098]   The invention concerns particularly the local treatment of infections due to herpesviridiae family virus.

[0099]   The invention composition is preferably used locally aiming to remove secondary effects, e.g., atherosclerosis. It can be applied to all external or internal mucous (e.g., oral,

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genital, vaginal, ophthalmic, otic, sinusal, nose-and-throat, dermal, and the like). The invention composition may appear under an adapted form for this administration, such as in a semi-liquid form (e.g., a gel) via an addition of one or several compatible pharmaceutical substances e.g., cellulose, amino acids, peptides, and/or proteins.

[0100]   The invention composition may also be adapted to clinical status and/or injured mucous. This adaptation is executed via a concentration change of active products of the therapeutic solutions.

[0101]   For non-restrictive examples of such adapted therapeutic solutions:

    i)   For an infection treatment.

[0102]   For endodontic treatment, concentrations between about 1 and about 0.2 moles/liter of sodium hypochlorite, and approximately between about 100 to about 0.001 picomoles/liter of TauCl are preferred (i.e., these concentrations vary with organic matter amount present in canals).

[0103] - With highly stained keratinized mucous (with profuse presence of organic matter (infectious agents, blood, profuse and varying secretions, suppurating discharge, etc.)), a sodium hypochlorite concentration between about 0.1 and about 0.02 mole/liter and a TauCl concentration between about 1 and about 0.001 picomoles/liter are preferable (non-restricting example).

[0104] - With moderately stained keratinized mucous (with some organic matter visible on a compress after a gentle friction, for example), a sodium hypochlorite concentration between about 20 and about 10 millimoles/liter of available chlorine and a TauCl concentration between about 1 and about 0.01 nanomole/liter are preferable (non-restricting example).

[0105] - With clean keratinized mucous (without organic matter visible), preferred concentrations may be between about 10 and about 2 millimoles/liter of available chlorine for

sodium hypochlorite (NaOCl), and between about 50 and about 1 micromoles/liter for TauCl (non-restricting example).

[0106] - With highly stained non-keratinized mucous, concentrations may be between about 50 and about 10 millimoles/liter of available chlorine for NaOCl and between about 0.1 and about 0.001 picomoles/liter for TauCl (non-restricting example).

[0107] - With moderately stained non-keratinized mucous, concentrations may be between about 10 and about 5 millimoles/liter of available chlorine for NaOCl and between about 1 and about 0.01 nanomoles/liter for TauCl (non-restricting example).

[0108] - With clean non-keratinized mucous, concentrations may be between about 5 and about 0.8 millimoles/liter of available chlorine for NaOCl and approximately between about 50 and about 1 micromoles/liter for TauCl (non-restricting example).

[0109] - With important and sensible organs (eyes), concentrations must both be the least toxic (via a high dilution or a specific scavenge of NaOCl by an antioxidant addition) and executed with a profuse diluted irrigation:

[0110] · For stained organs, concentrations may be between about 5 and about 0.1 millimoles/liter of available chlorine for NaOCl and between about 1 and about 0.01 femtomoles/liter for TauCl (non-restricting example).

[0111] · For non-stained organs, concentrations may be between about 0.1 and about 0.01 millimoles/liter of available chlorine for NaOCl and between about 50 and about 1 micromoles/liter for TauCl (non-restricting example).

ii) For the treatment of non-stained organs with the object of immune stimulation and/or tissue regeneration, concentrations could be between about 500 and about 1 micromoles/liter of available chlorine for NaOCl and between about 200 and about 10 micromoles for TauCl (non-restricting example).

[0112]   The composition according to the invention is useful for local treatment of diseases or inflammatory processes that can be chronic, and/or progressive and/or acute. The composition is also recommended for pre- surgical irrigation and/or per-surgical irrigation and/or post-surgical irrigation of internal and/or external mucous and of opened-injures. The invention more particularly concerns a treatment method of lesions and infections described above, which comprises contacting the invention composition on mucous that must be treated, (for non-restricting example) between 2 and 3 times a day and approximately during 20 to 60 seconds, not followed by a rinsing. The composition amount employed should be sufficient to not generate a total neutralization of the therapeutic active agents. In the therapeutic use, the invention solution should not stay static. Concentrations of the composition invention should be adapted to the evolution of the clinical status until healing.

[0113]   The invention more particularly concerns the local treatment of lesions and infections linked to chronic and/or acute parodontitis. Thus, the invention composition is usefully adapted for irrigation of periodontal pockets, with the aim for removing these periodontal pockets as the composition has both antiseptic and anti-inflammatory activities, and acts as an immunity modulator and healing stimulator of periodontal tissues (i.e., alveolar bone, alveolodental ligament and gingiva).

[0114]   Chronic periodontitis is a disease mainly due to pathologic action of anaerobic bacteria, and particularly Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides forsythus and Prevotella intermedia. These bacteria induce chronic inflammatory processes that generate a progressive destruction of periodontal tissues (teeth supporting tissue). Periodontitis may result in the removal of bone tissue followed by tooth loss.

[0115]   Whatever the treatment phase of chronic periodontitis, periodontal pocket irrigations have to be executed in the presence of a strong surgical vacuum extraction with the object of avoiding swallowing or inhalation of the therapeutic solution by the patient.

[0116] i)   Attack treatment (i.e., between two and three weeks up to the disappearance of bleeding in probing depth of periodontal pockets).

[0117] - J 1:   after an assessment of the clinical status, crevicular spaces (with or without periodontal pockets) of oral cavity teeth should be irrigated. A full mouth followed by a tongue brushing, with a mixed solution of 0.1% chlorhexidine and 0.3% hydrogen peroxide, should be prescribed twice a day (far from the teeth brushing) over ten days, then twice to three times a week ad vitam aeterman (however, in halitosis, the initial attack treatment should be repeated). Two or three appointments should be scheduled.

[0118] - In the other sessions, the following process will be recommended: education, checking, and motivation for periodontal hygiene; meticulous irrigation (1 ml minimum of the highly stained keratinized mucous solution for each periodontal site); meticulous scaling and root planning.

[0119] - When all root surfaces are planned and cleaned, a probing session (this consists first of an   irrigation followed by a probing depth) should be executed to evaluate the degree of periodontal disease. Some complementary examinations can be made such as sampling picks up and biological examinations.

    ii)   Primary curative treatment (i.e., four weeks).

[0120]   Meticulous irrigation of periodontal pockets once every ten days applying the solution for moderately stained keratinized mucous, except for sites with a profuse dental plaque (such as interdental furcations) where the solution for highly stained keratinized mucous should be applied.

[0121]   At the last session of the primary curative treatment, an irrigation follows probing depth and root planning.

iii)   Secondary curative treatment (until clinical removal of the periodontal pockets).

[0122]   Meticulous irrigation of periodontal pockets once every ten days with the solution for clean keratinized mucous, except for sites with + or – profuse dental plaque (e.g., interdental furcations) where the therapeutic composition for highly or moderate stained keratinized mucous should be used.

[0123]   Every three sessions of the secondary curative treatment, an irrigation follows probing depth of periodontal pockets and root planning.

iv)   Maintenance treatment.

[0124]   In any diagnostic of clinical healing, a maintenance treatment should be executed. This treatment type is similar to the secondary curative treatment except that appointments should be made once every three weeks.

[0125]   If after two mouths of treatment a notable healing rather than a recurrence is observed, the last treatment phase – supervision – can be initiated.

[0126]   In case of recurrence, the treatment should be started again at a stage that depends on the clinical status observed, i.e., the attack treatment, or the primary or the secondary curative treatment.

v)   Supervision.

[0127]   An appointment should be made once every six weeks. A meticulous probing depth will be practiced.

[0128] - In case of no-recurrence, all crevicular spaces should be irrigated with the solution for moderate stained or clean keratinized mucous, followed by a meticulous root planning.

[0129] - In case of recurrence, the treatment should be started again at a stage that depends on the clinical status observed (i.e., the attack treatment, or the primary or the secondary curative treatment).

[0130]   In addition, the invention also concerns bone-filling surgical periodontal treatments with some biomaterials associated with the invention composition and/or one of its components.

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